Citation:

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Study Design:

Trend study

Class:

C - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

POSITIVE: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To examine how population concentrations of serum and red blood cell (RBC) folate and serum vitamin B_{12} changed over the past twenty years.

Inclusion Criteria:

- Participants in the pre-fortification third National Health and Nutrition Examination Survey (NHANES III; 1988 to 1994)
- Participants in three post-fortification NHANES periods (covering 1999 to 2004).

Exclusion Criteria:

No participants were excluded from the analyses.

Description of Study Protocol:

Recruitment

- NHANES conducted nationally representative cross-sectional probability surveys of the non-institutionalized civilian population of the United States. The participants obtained a stratified, multistage probability sample to represent the US population according to age, gender and race or ethnicity
- During each survey period, certain sub-populations were over-sampled to allow for more precise estimates. The procedures for NHANES 1999 to 2000, 2001 to 2002 and 2003 to 2004 were similar to those used for NHANES III (1988 to 1994).

Design

- Time series: Repeated population-based cross-sectional surveys
- The analysis of variance model included:
    - Four age groups:
        - Four to 11 years (children)
        - 12 to 19 years (adolescents)
        - 20 to 59 years (adults)
        - 60 years or older (older persons)
    - Gender and raceor ethnicity categories:
        - Non-Hispanic white (NHW)
        - Non-Hispanic black (NHB)
        - Mexican American (MA).

Dietary Intake/Dietary Assessment Methodology

Folic acid and vitamin B_{12} status was measured using the serum and RBC folate and serum vitamin B_{12} concentrations.

Blinding Used

Blood indicators of folate and vitamin B_{12} were measured using laboratory tests.

Intervention

Folic acid fortification policy.

Statistical Analysis

- Statistical analyses were performed by SAS (version 9; SAS Institute Inc, Cary, NC) and SUDAAN (version 9; RTI, Research Triangle Park, NC) software
- Sample weights were used to account for differences in non-response or non-coverage and to adjust for planned oversampling of some groups
- 95% CI was estimated with SUDAAN using Taylor series linearization
- Length of fasting (less than one hour to 12 hours or more) had no big influence on serum folate concentrations, no matter the session (morning, afternoon or evening) in which participants were examined
- Frequency distributions for serum and RBC folate concentrations were used to illustrate changes in the distribution of folate concentrations over time
- Medians or geometric means (log10-transformed data) were evaluated due to their skewed distributions
- Prevalence estimates (percent) and 95% CIs of subjects at risk of low or high concentrations of folate and vitamin B_{12} were determined for specific groups of public health interest, e.g., women of childbearing age (15 to 45 years old), children and older persons
- Sex X survey period, race X survey period and age X survey period interactions were tested using an analysis of variance model that included age (four age groups), sex (male or female), racial or ethnic group (NHW, NHB, MA or other) and the interaction terms
- Geometric means were tested for significant differences in subgroup analysis since no satisfactory parametric approach existed for a statistical analysis of complex survey data that compares medians
- Time trend analysis (using a two-tailed, two-group T-test) was restricted to age-specific subgroups (four to 11, 12 to 19, 20 to 59, and 60 years or older) due to the age X survey period interaction
- To adjust for multiple comparisons, the P value of each comparison was considered significant if it was 0.017 (0.05 divided by three, the total number of comparisons)
- Selected population percentile values (2.5th, 50th and 97.5th) and their 95% CI for serum and RBC folate and for serum vitamin B_{12} were also determined.

Data Collection Summary:

Timing of Measurements

Population-based survey covering pre-fortification period 1988 to 1994 (six years) and the post-fortification period 1999 to 2004 (six years).

Dependent Variables

Folate for 1988 to 1994 and 1999 to 2004; vitamin B_{12} for 1991 to 1994 and 1999 to 2004; and RBC folate for 1988 to 1994 and 1999 to 2004:

- Conducted on samples of venous serum or whole blood hemolysate
- Radioassay (Quantaphase I; BioRad, Hercules, CA) used for the serum and RBC folate measurements in NHANES 1988 to 1991; Quantaphase II was used in 1991 to 2004 for serum and RBC folate and for serum vitamin B_{12} measurements
- Adjustments made to NHANES 1988 to 1991 folate data before public release to account for method differences between the Quantaphase I and II and to make the data comparable to those from NHANES 1991 to 1994
- Well-characterized quality-control pools also used to ensure unbiased results over time
- Long-term CVs for each two-year post-fortification period were 4% to 7% for serum folate at 2.30 to 13.2ng per ml, 3% to 6% for serum vitamin B_{12} at 381 to 1,570pg per ml and 4% to 6% for RBC folate at 63 to 494ng per ml

- Cutoffs used were less than 3.0ng per ml for low serum folate, less than 140ng per ml for low RBC folate and less than 200pg per ml for low serum vitamin B_{12}. The cutoff for high serum folate was 20ng per ml.

Independent Variables

The implementation of a folic acid fortification program in the mid-1990s.

Description of Actual Data Sample:

- *Initial N:* All participants from NHANES III (1988 to 1994) and post-fortification NHANES periods (1999 to 2004)
- *Attrition (final N):* About 23,000 participants in NHANES III and about 8,000 participants in post-fortification NHANES periods (1999 to 2004) were included in the analyses
- *Ethnicity:* Non-Hispanic white (NHW), non-Hispanic black (NHB) and Mexican American (MA)
- *Location:* United States.

Summary of Results:

TABLE 1. Trends in Biochemical Folate and Vitamin B_{12} Concentrations in the US Population by Sex, Race or Ethnicity and Age Group During the National Health and Nutrition Examination Survey (NHANES), 1988–2004[1]

			Median (95% CI)				Pairwise Comparison (T-test) of Geometric Means (P[2])		
Race/ethnicity	**Sex**	**Age group**	**1988–1994[3]**	**1999–2000**	**2001–2002**	**2003–2004**	**1988–1994 vs. 1999–2000[4]**	**1999–2000 vs. 2001–2002[5]**	**2001–2002 vs. 2003–2004[6]**
Serum folate (ng per ml)[7]		Y							
All	Both	All	5.5 (5.2, 5.7)	14.1 (13.3, 14.9)	13.0 (12.5, 13.4)	11.9 (11.4, 12.3)	—	—	—
All	M	All	5.3 (5.0, 5.5)	13.3 (12.5, 14.2)	12.5 (12.0, 12.9)	11.5 (11.0, 11.9)	—	—	—
All	F	All	5.7 (5.5, 6.0)	14.7 (14.0, 15.9)	13.5 (13.1, 13.9)	12.5 (11.9, 12.9)	—	—	—
NHW	Both	All	5.8 (5.5, 6.1)	14.8 (13.8, 16.2)	13.5 (13.0, 14.0)	12.6 (12.0, 13.3)	—	—	—
NHB	Both	All	4.6 (4.4, 4.7)	11.6 (10.9, 12.3)	10.8 (10.1, 11.3)	10.1 (9.30, 10.8)	—	—	—
MA	Both	All	5.1 (4.7, 5.3)	13.4 (12.8, 13.9)	11.7 (10.9, 12.5)	11.1 (10.7, 11.6)	—	—	—
All	Both	4-11	8.8 (8.3, 9.3)	19.3 (18.5, 19.9)	17.0 (16.4, 17.7)	15.6 (15.0, 16.3)	<0.001	<0.001	0.01
All	Both	12-19	5.1 (4.9, 5.5)	13.3 (12.7, 14.0)	12.6 (11.9, 13.3)	11.0 (10.4, 11.4)	<0.001	0.014	0.002
All	Both	20-59	4.8 (4.5, 5.0)	12.5 (11.6, 13.6)	11.8 (11.2, 12.2)	11.0 (10.5, 11.3)	<0.001	NS	0.011
All	Both	60 years or older	6.9 (6.6, 7.3)	17.4 (16.4, 18.2)	16.6 (15.4, 17.5)	15.6 (14.8, 16.4)	<0.001	0.009	NS

Red blood cell folate (ng per ml)									
All	Both	All	174 (169, 180)	276 (266, 289)	274 (265, 282)	254 (246, 262)	—	—	—
All	M	All	172 (166, 176)	270 (258, 280)	265 (254, 276)	248 (239, 258)	—	—	—
All	F	All	177 (169, 184)	286 (272, 299)	281 (273, 291)	262 (251, 271)	—	—	—
NHW	Both	All	184 (177, 189)	293 (275, 308)	287 (282, 295)	268 (257, 279)	—	—	—
NHB	Both	All	135 (132, 139)	226 (219, 232)	214 (209, 220)	210 (205, 214)	—	—	—
MA	Both	All	164 (156, 170)	255 (250, 261)	252 (240, 264)	236 (230, 244)	—	—	—
All	Both	4-11	200 (190, 210)	287 (279, 292)	274 (263, 284)	256 (247, 266)	<0.001	NS	0.002
All	Both	12-19	150 (143, 156)	244 (237, 256)	237 (226, 248)	224 (215, 230)	<0.001	NS	0.003
All	Both	20-59	164 (160, 171)	269 (256, 285)	271 (260, 280)	248 (239, 258)	<0.001	NS	<0.001
All	Both	60 years or older	211 (204, 220)	344 (323, 363)	335 (325, 349)	321 (310, 337)	<0.001	NS	NS
Serum vitamin B_{12} (pg per ml)									
All	Both	All	466 (453, 478)	482 (472, 492)	483 (472, 492)	483 (466, 499)	—	—	—
All	M	All	464 (449, 479)	484 (474, 497)	483 (467, 495)	483 (468, 497)	—	—	—
All	F	All	466 (449, 484)	476 (465, 493)	484 (473, 493)	484 (462, 504)	—	—	—
NHW	Both	All	444 (427, 460)	465 (452, 476)	471 (458, 485)	466 (447, 486)	—	—	—
NHB	Both	All	567 (553, 579)	578 (566, 597)	551 (535, 566)	545 (520, 581)	—	—	—
MA	Both	All	495 (471, 522)	525 (502, 539)	492 (465, 529)	517 (496, 535)	—	—	—
All	Both	4-11	667 (636, 703)	710 (677, 747)	714 (687, 745)	724 (708, 748)	NS	NS	NS
All	Both	12-19	480 (464, 507)	506 (494, 518)	515 (496, 537)	504 (481, 528)	NS	NS	NS
All	Both	20-59	436 (423, 456)	447 (438, 457)	449 (439, 459)	452 (435, 470)	NS	NS	NS
All	Both	60 years or older	413 (398, 430)	469 (458, 482)	480 (465, 493)	477 (453, 494)	<0.001	NS	NS

- Serum and red blood cell folate concentrations were measured in ng per ml; serum vitamin B_{12} concentrations were measured in pg per ml.
- SI conversion: To convert serum folate to nmol per L, multiply by 2.266; to convert red blood cell folate to nmol per L, multiply by 2.266; to convert serum vitamin B_{12} to pmol per L, multiply by 0.7378
- For serum and red blood cell folate, time trend analysis of groups comprising the entire age range could not be performed because of a significant age X survey period interaction. For serum vitamin B_{12}, time trend analysis of groups comprising the entire age range and all races could not be performed because of significant age X survey period and race X survey period interactions.
- In NHANES III, serum vitamin B_{12} concentrations were measured only for all persons aged four years or older in 1991 to 1994
- Folate measurements were performed by using the Quantaphase II radioassay (BioRad Diagnostics, Hercules, CA), which measures, on average, about 35% lower than does the microbiologic assay.

TABLE 2. Trends in the Prevalence of the Risk of Low or High Blood Folate and Vitamin B_{12} Concentrations in the Entire US Population and in Groups of Special Public Health Interest During the National Health and Nutrition Examination Survey (NHANES), 1988–2004[1]

			Prevalence (95% CI)				Pairwise Comparison (T-test) of Prevalence Estimates (P)		
Race/ethnicity	**Sex**	**Age Group**	**1998-1994[2]**	**1999–2000**	**2001–2002**	**2003–2004**	**1988–1994 vs. 1999–2000[3]**	**1999–2000 vs. 2001–2002[4]**	**2001–2002 vs. 2003–2004[5]**
Serum folate less than 3ng per ml		y							
All	F	15-45	20.6 (18.6, 22.8)	0.8 (0.3, 1.7)	0.3 (0.1, 1.0)	0.6 (0.2, 1.5)	<0.001	NS	NS
NHW	F	15-45	20.0 (17.4, 22.8)	1.1 (0.5, 2.4)	0.4 (0.1, 1.6)	0.7 (0.2, 2.1)	<0.001	NS	NS
NHB	F	15-45	29.7 (27.6, 31.9)	0.4 (0.1, 2.7)	0.4 (0.1, 1.8)	0.8 (0.2, 3.2)	<0.001	NS	NS
MA	F	15-45	21.7 (18.6, 25.2)	0.0	0.4 (0.1, 2.3)	0.3 (0.0, 2.4)	<0.001	NS	NS
Red blood cell folate less than 140ng ml									
All	F	15-45	37.6 (34.4, 40.9)	5.1 (3.4, 7.5)	4.0 (2.6, 5.9)	5.5 (4.5, 6.6)	<0.001	NS	NS
NHW	F	15-45	34.5 (30.6, 38.6)	4.4 (2.3, 8.4)	2.7 (1.3, 5.7)	3.5 (2.7, 4.6)	<0.001	NS	NS
NHB	F	15-45	59.6 (56.5, 62.6)	11.9 (7.6, 18.3)	14.1 (9.7, 20.1)	14.3 (10.8, 18.8)	<0.001	NS	NS
MA	F	15-45	38.7 (33.4, 44.2)	1.6 (0.6, 3.8)	2.8 (1.4, 5.7)	4.7 (3.0, 7.5)	<0.001	NS	NS
Serum folate more than 20ng per ml									
All	Both	4-11	5.4 (3.7, 7.6)	42.4 (35.7, 49.3)	26.8 (23.4, 30.6)	18.6 (15.2, 22.5)	<0.001	<0.001	0.002
All	Both	60 years or more	7.1 (5.8, 8.8)	38.0 (34.5, 41.7)	31.5 (28.4, 34.9)	31.8 (28.2, 35.6)	<0.001	0.009	NS

All	M	60 years or more	5.0 (3.6, 6.9)	29.2 (25.2, 33.6)	25.3 (22.0, 28.9)	27.5 (22.8, 32.8)	<0.001	NS	NS
All	F	60 years or more	8.8 (7.0, 11.1)	45.2 (41.2, 49.3)	36.2 (31.6, 41.0)	35.1 (31.5, 38.9)	<0.001	0.005	NS
Serum vitamin B_{12} less than 200pg per ml									
All	Both	60 years or more	4.8 (3.6, 6.2)	2.7 (2.0, 3.7)	3.6 (2.8, 4.6)	3.9 (2.9, 5.2)	0.012	NS	NS
All	M	60 years or more	5.2 (3.7, 7.4)	2.6 (1.7, 4.0)	3.4 (2.6, 4.5)	3.8 (2.4, 5.9)	NS	NS	NS
All	F	60 years or more	4.4 (2.9, 6.5)	2.8 (1.9, 4.1)	3.7 (2.3, 5.7)	4.0 (2.4, 6.4)	NS	NS	NS
NHW	Both	60 years or more	5.1 (3.8, 6.8)	2.7 (1.8, 4.1)	3.6 (2.8, 4.6)	4.1 (3.1, 5.3)	NS	NS	NS
NHB	Both	60 years or more	1.8 (0.9, 3.7)	1.4 (0.6, 3.2)	1.0 (0.3, 3.3)	2.0 (1.0, 3.9)	NS	NS	NS
MA	Both	60 years or more	6.3 (4.0, 9.8)	4.0 (2.0, 7.8)	1.9 (0.6, 5.9)	1.7 (1.1, 2.8)	NS	NS	NS

TABLE 4. Selected Population Percentile Values for Biochemical Folate and Vitamin B_{12} Concentrations by Sex, Race or Ethnicity, and Age Group During the National Health and Nutrition Examination Survey (NHANES), 1988–1994 and 1999–2004[1]

					Percentile (95% CI)		
Race/ethnicity	**Sex**	**Age Group**	**Survey**	**Subjects**	**2.5th**	**50th**	**97.5th**
		Y		**N**			
Serum folate[2]							
All	Both	All	1988–1994	23,361	1.7 (1.6, 1.8)	5.5 (5.2, 5.7)	21.3 (19.9, 22.6)
			1999–2004	23,345	4.6 (4.5, 4.9)	13 (12.7, 13.2)	34.8 (34.1, 36.0)
All	M	All	1988–1994	11,130	1.8 (1.6, 1.9)	5.3 (5.0, 5.5)	18.6 (17.6, 20.2)
			1999–2004	11,387	4.5 (4.3, 4.7)	12.3 (12.0, 12.7)	32.2 (31.2, 33.6)

All	F	All	1988–1994	12,231	1.7 (1.6, 1.8)	5.7 (5.5, 6.0)	23.2 (21.6, 25.7)
			1999–2004	11,958	4.8 (4.6, 5.1)	13.6 (13.2, 13.9)	37.3 (35.7, 39.5)
NHW	Both	All	1988–1994	8,534	1.7 (1.6, 1.8)	5.8 (5.5, 6.1)	22.4 (20.6, 24.2)
			1999–2004	9,427	4.9 (4.6, 5.1)	13.6 (13.1, 13.9)	36.5 (35.0, 37.8)
NHB	Both	All	1988–1994	6,813	1.6 (1.5, 1.6)	4.6 (4.4, 4.7)	16.3 (15.3, 16.8)
			1999–2004	5,764	4.1 (3.8, 4.4)	10.7 (10.4, 11.1)	30.1 (27.7, 32.8)
MA	Both	All	1988–1994	7,017	1.7 (1.7, 1.8)	5.1 (4.7, 5.3)	17.0 (15.1, 19.3)
			1999–2004	6,500	4.7 (4.2, 4.9)	11.9 (11.4, 12.2)	28.7 (27.2, 30.6)
All	Both	4-11	1988–1994	4,627	3.3 (3.1, 3.6)	8.8 (8.3, 9.3)	26.9 (22.6, 29.5)
			1999–2004	3,595	8.6 (8.2, 9.0)	17.2 (16.9, 17.6)	37.7 (34.7, 41.0)
All	Both	12-19	1988–1994	2,957	1.7 (1.6, 1.9)	5.1 (4.9, 5.5)	16.1 (14.3, 18.1)
			1999–2004	6,390	5 (4.8, 5.2)	12.1 (11.7, 12.5)	27.2 (25.6, 28.2)
All	Both	20-59	1988–1994	10,726	1.6 (1.4, 1.6)	4.8 (4.5, 5.0)	18.5 (17.1, 20.4)
			1999–2004	8,689	4.4 (4.1, 4.5)	11.6 (11.3, 11.9)	31.0 (30.1, 32.6)
All	Both	60 years or more	1988–1994	5,051	2.1 (1.9, 2.3)	6.9 (6.6, 7.3)	28.1 (25.2, 30.3)
			1999–2004	4,671	5.6 (5.2, 5.9)	16.6 (16.0, 17.0)	45.8 (43.7, 48.4)
Red blood cell folate[2]							
All	Both	All	1988–1994	23,402	77 (74, 79)	174 (169, 180)	438 (423, 456)
			1999–2004	23,527	135 (131, 138)	269 (263, 274)	600 (581, 618)
All	M	All	1988–1994	11,144	79 (74, 82)	172 (166, 176)	412 (393, 439)
			1999–2004	11,455	136 (131, 140)	260 (255, 266)	566 (545, 580)
All	F	All	1988–1994	12,258	75 (71, 77)	177 (169, 184)	455 (439, 473)
			1999–2004	12,072	132 (128, 136)	276 (272, 282)	630 (598, 657)
NHW	Both	All	1988–1994	8,574	79 (76, 84)	184 (177, 189)	455 (437, 473)

			1999–2004	9,472	145 (138, 150)	284 (277, 290)	636 (608, 657)
NHB	Both	All	1988–1994	6,869	58 (53, 60)	135 (132, 139)	317 (305, 330)
			1999–2004	5,856	111 (108, 114)	216 (213, 219)	433 (411, 462)
MA	Both	All	1988–1994	6,956	79 (74, 82)	164 (156, 170)	368 (342, 401)
			1999–2004	6,526	135 (129, 141)	247 (242, 252)	519 (494, 539)
All	Both	4-11	1988–1994	4,660	101 (95, 107)	200 (190, 210)	386 (358, 409)
			1999–2004	3,656	172 (164, 176)	271 (266, 275)	507 (473, 523)
All	Both	12-19	1988–1994	2,955	72 (67, 76)	150 (143, 156)	360 (332, 384)
			1999–2004	6,425	131 (127, 135)	235 (230, 240)	454 (425, 490)
All	Both	20-59	1988–1994	10,720	74 (70, 77)	164 (160, 171)	414 (395, 433)
			1999–2004	8,732	131 (127, 134)	263 (256, 269)	567 (539, 595)
All	Both	60 years or more	1988–1994	5,067	83 (80, 88)	211 (204, 220)	573 (525, 611)
			1999–2004	4,714	145 (140, 152)	333 (325, 344)	744 (718, 789)
Serum vitamin B_{12}[3]							
All	Both	All	1991–1994	11,860	196 (181, 212)	466 (453, 478)	1,070 (1020, 1120)
			1999–2004	23,346	209 (206, 212)	483 (475, 490)	1,180 (1150, 1210)
All	M	All	1991–1994	5,380	200 (179, 220)	464 (449, 479)	1,050 (993, 1100)
			1999–2004	11,381	219 (212, 226)	484 (476, 491)	1,100 (1080, 1140)
All	F	All	1991–1994	6,480	196 (179, 214)	466 (449, 484)	1,090 (1030, 1160)
			1999–2004	11,965	202 (199, 207)	482 (473, 491)	1,260 (1200, 1300)
NHW	Both	All	1991–1994	3,989	200 (183, 213)	444 (427, 460)	986 (918, 1070)
			1999–2004	9,430	203 (201, 208)	469 (460, 476)	1,100 (1080, 1140)
NHB	Both	All	1991–1994	3,903	237 (218, 260)	567 (553, 579)	1,280 (1200, 1360)
			1999–2004	5,762	238 (226, 252)	561 (547, 574)	1,340 (1310, 1390)

MA	Both	All	1991–1994	3,365	205 (193, 228)	495 (471, 522)	1,290 (1160, 1470)
			1999–2004	6,499	234 (219, 243)	513 (498, 524)	1,450 (1240, 1760)
All	Both	4-11	1991–1994	2,204	346 (321, 377)	667 (636, 703)	1,350 (1250, 1460)
			1999–2004	3,594	337 (326, 350)	715 (700, 732)	1,470 (1380, 1540)
All	Both	12-19	1991–1994	1,593	248 (224, 274)	480 (464, 507)	1,010 (909, 1150)
			1999–2004	6,390	236 (228, 243)	508 (497, 520)	1,050 (1000, 1080)
All	Both	20-59	1991–1994	5,556	188 (172, 207)	436 (423, 456)	935 (870, 1030)
			1999–2004	8,692	207 (203, 211)	451 (443, 458)	1,060 (1010, 1100)
All	Both	60 years or more	1991–1994	2,507	158 (145, 179)	413 (398, 430)	1,060 (977, 1200)
			1999–2004	4,670	184 (168, 194)	474 (467, 483)	1,250 (1170, 1330)

Other Findings

Frequency distributions for serum vitamin B_{12} for the entire population for each of the four survey periods did not appear to change significantly (data not shown).

Frequency distributions for serum and RBC folate:

- There was a remarkable upward shift in the entire distribution of serum and RBC folate concentrations from before fortification to the first post-fortification survey period
- There was an apparent decline mainly at the upper end of the distribution in the two most recent post-fortification survey periods with no apparent decline at the lower end of the distribution.

The lowest post-fortification distributions of serum and RBC folate concentrations were seen for 2003 to 2004. The distribution curve for that survey period was still much higher than that for the pre-fortification survey.

Author Conclusion:

- The decrease in folate concentrations observed longer after fortification was small, compared with the increase soon after the introduction of fortification
- The decrease was not at the low end of concentrations and therefore, did not raise concerns about inadequate status.

Reviewer Comments:

- *The authors argued that from the perspective of nutritional adequacy relative to generally accepted nutritional status criteria, the slight downward trend after fortification was unlikely to be functionally important*
- *The authors argued that the recent decreases in blood folate concentrations might be due in part to changes in consumer behaviors*
- *The current paper suggested that the concentration changes emphasized the need for further monitoring of the fortified-food supply, as well as blood concentrations.*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions

1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions

1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes
2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	N/A
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	N/A
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	N/A
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	N/A
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	N/A
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	N/A
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes

4.4.	Were reasons for withdrawals similar across groups?	N/A
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes
5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	N/A
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	Yes
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	N/A
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	Yes
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	???
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	No
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	Yes
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	N/A
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	Yes
7.6.	Were other factors accounted for (measured) that could affect outcomes?	Yes
7.7.	Were the measurements conducted consistently across groups?	Yes
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	Yes
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	Yes
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A

	8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	Yes
	8.6.	Was clinical significance as well as statistical significance reported?	Yes
	8.7.	If negative findings, was a power calculation reported to address type 2 error?	N/A
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**		Yes
	9.1.	Is there a discussion of findings?	Yes
	9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**		Yes
	10.1.	Were sources of funding and investigators' affiliations described?	Yes
	10.2.	Was the study free from apparent conflict of interest?	Yes